In vitro and in vivo mechanistic studies toward understanding the role of 1-aminobenzotriazole in rat drug-drug interactions

Marc-Olivier Boily, Nathalie Chauret, Julie Laterreur, François A. Leblond, Chantal Boudreau, Marie-Claude Duquet, Jean-François Lévesque, Line Ste-Marie, Vincent Pichette

Service de Néphrologie et Centre de Recherche, Hôpital Maisonneuve-Rosemont, Faculté de Médecine, Université de Montréal, Québec, Canada. MOB, FAL, VP

Vertex Pharmaceuticals Canada Incorporated, Laval, Quebec, Canada. MOB, NC, JL, CB, MCD, JFL, LSM
Running title: Effect of ABT on pharmacokinetics of metoprolol in rats

Corresponding author:

Marc-Olivier Boily
Labo 35, Centre de recherche, Hôpital-Maisonneuve Rosemont
5415 boul. de l’assomption
Montréal, Québec, Canada, H1T 2M4
819-238-3943
Marc-Olivier.Boily@usherbrooke.ca

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Abbreviations: ABT, 1-Aminobenzotriazole; AUC, Area under the curve; CE, Collision energy; CL_{int}, Intrinsic clearance; Cmax, Maximal concentration; DDI, Drug-drug interactions; % F, Bioavailability; i.v., Intravenous; Km, Concentration at half the maximal velocity; LC-MS/MS, Liquid chromatography coupled with tandem mass spectrometry; MA, Metoprolol acid; ODM, o-desmethyl-metoprolol; OHM, α-hydroxy-metoprolol; PK, Pharmacokinetics; T_{1/2}, Half-life; Tmax, Time at maximal concentration; Vmax, Maximal velocity.
Abstract

1-Aminobenzotriazole (ABT) is regularly used in vivo as a non-specific and irreversible cytochrome P450 inhibitor to elucidate the role of metabolism on the pharmacokinetic profile of xenobiotics. However, few reports have considered the recent findings that ABT can alter drug absorption or have investigated the possible differential inhibition of ABT on intestinal and hepatic metabolism. To address these uncertainties, pharmacokinetic studies under well-controlled and defined ABT pretreatment conditions (50 mg/kg, 1 h ABT i.v. and 16 h ABT p.o.) were conducted prior to the oral administration of metoprolol, a permeable P450 probe that undergoes extensive intestinal and hepatic metabolism. The pharmacokinetic profile of metoprolol was affected differently by the two ABT pretreatments. An increase in AUC of 16-fold with ABT p.o. and 6.5-fold with ABT i.v. was observed compared to control. Based on in vitro studies, this difference could not be attributed to a differential inhibition of intestinal and hepatic metabolism. In the ABT i.v. pretreatment, the increase in AUC was also associated with a prolonged Tmax (24-fold versus control) suggesting a delay in absorption. This was further confirmed by the administration of a charcoal meal which resulted in a 7-fold increase in stomach weights in the 1 h ABT pretreated groups compared to untreated or 16 h ABT pretreated rats. Based on these results, we recommend pretreating rats with ABT p.o. 16 h before the administration of a test compound to preserve the inhibitory effect on intestinal and hepatic metabolism and to avoid the confounding effect on drug absorption.
Introduction

1-Aminobenzotriazole (ABT) is an irreversible and non-specific inhibitor of the major cytochrome P450 enzymes responsible for the metabolism of many xenobiotics (Balani et al., 2002). Upon oxidation, ABT forms a reactive benzyne intermediate which inactivates the cytochrome P450 by alkylation of the protesthetic heme (Mico et al., 1988). Since ABT is highly soluble, has a good in vivo bioavailability (%F), a low clearance (Town et al., 1993) and a good safety profile at doses up to 100 mg/kg in rats (Meschter et al., 1994), it is used to understand the role of metabolism on the pharmacokinetics (PK) of drugs in pre-clinical species. For example, it has been reported that ABT can be used to discriminate between poor absorption and extensive CYP-mediated metabolism for poorly bioavailable compounds (Caldwell et al., 2005). It can also be valuable in distinguishing between the toxicity mediated by the parent drug and its metabolites (Subramanian et al., 2014). There is even some evidence that the route of administration of ABT could provide insights on the contribution of hepatic versus intestinal metabolism to the first pass effect. This assumption is predicated on the fact that the bioavailability of midazolam was not affected by a treatment with ABT i.v., while there was a significant increase when ABT was given orally. Since midazolam is known to be highly metabolised in the gut, the authors concluded that ABT i.v. did not inhibit gut enzymes in comparison to ABT p.o. (Strelevitz et al., 2006).

Although there are numerous studies demonstrating the ability of ABT to block metabolism in vivo, there is a lack of consistent methodologies in these studies (Table 1) and therefore, it is difficult to extrapolate conclusions from one study to another. In addition, most studies report changes in the pharmacokinetic profiles of the probe substrates but few have examined the corresponding changes in the pharmacokinetic profiles of the circulating metabolites. This may lead to misinterpretation of the impact of ABT on in vivo metabolism, in light of the recent report that ABT may also affect intestinal transit time (Stringer et al., 2014).
Finally, none of these studies have undertaken a systematic approach to measure the impact of ABT pretreatment on the role of gut and liver metabolism.

The aim of the present study is to define the best pretreatment conditions for the use of ABT in rats to avoid any disturbance in the gastro-intestinal tract while maintaining its P450 inactivation. Metoprolol was selected as the P450 probe for these studies. Metoprolol is completely absorbed but exhibits very poor bioavailability (F of 4%) due to high first pass metabolism (Borg et al., 1975b) in the intestine and liver (Yoon et al., 2011). The metabolic pathways of metoprolol are represented in Figure 1. Pharmacokinetic studies with metoprolol were conducted in rats treated with ABT under well-controlled and defined dosing conditions (50 mg/kg, 1 h ABT i.v. pretreatment and 16 h p.o. pretreatment). To obtain the complete absorption and metabolic profile of metoprolol, the plasma levels of three major circulating metabolites of metoprolol (α-hydroxy-metoprolol (OHM), o-desmethyl-metoprolol (ODM) and metoprolol acid (MA) (Borg et al., 1975a)) were also measured. In addition, to put into perspective the impact of ABT on metoprolol intestinal and hepatic metabolism, in vitro metabolic studies using intestinal and hepatic microsomes prepared from ABT pretreated rats were conducted. Finally the impact of ABT on the gastric emptying and the intestinal transit time was assessed using the charcoal method. Based on our results, we provide recommendations for the future use of ABT in pharmacokinetics studies.

Materials and methods

Drugs and chemicals.

Metoprolol tartrate salt, labetalol hydrochloride, activated charcoal and reduced β-NADPH sodium salt were purchased from Sigma Aldrich (St Louis, MO, United-States). Metoprolol acid (MA), α-hydroxy-metoprolol (OHM) and o-desmethylmetoprolol (ODM) were
obtained from Toronto Research Chemicals (Toronto, On, Canada). All other reagents were of ACS grade or higher.

**Animals.**

Male Sprague-Dawley rats weighing 200-250 g were obtained from Charles River (St-Constant, Quebec, Canada) and were housed in a temperature-controlled room with a 12 h light-dark cycle. All rats were fasted 15-16 h prior to experiments and water was available *ad libitum*. Oral administrations were performed by delivering a bolus of the drug in solution directly into the stomach using a syringe with a flexible gavage needle. All i.v. administrations were made by injecting a bolus of the drug solution via a tail vein. For pharmacokinetic studies, standard rat chow was provided 2 h post administration of the drug. All the experiments were approved by an ethical committee and were conducted according to the Canadian Council on Animal Care guidelines for the care and use of laboratory animals.

**Pharmacokinetics studies.**

To assess the impact of ABT on the pharmacokinetics of metoprolol, metoprolol was formulated in water and dosed p.o. at 10 mg/kg in control rats and rats pretreated 1 h with ABT i.v. (50mg/kg in saline, 1mL/kg) or 16 h with ABT p.o. (50mg/kg in saline, 5mL/kg) n=5 per group, see *Table 2* for specific conditions. Blood was collected from the jugular vein at 0.25, 0.5, 1, 2, 4, 9, 8 and 24 hrs post dosing. Samples were immediately transferred into tubes containing EDTA K3 and plasma was obtained following a centrifugation at 1620 g for 10 min at 4°C. The plasma samples were stored at -30°C until analysis.

**Preparation of hepatic and intestinal microsomes.**

To determine the effect of ABT on hepatic and intestinal enzymes, untreated as well as ABT i.v. and p.o. pretreated rats (see *Table 2* for details) were anesthetized with isoflurane and
sacrificed by cervical dislocation (n=3 per group). The livers were immediately collected, cut into pieces of 1-5 mm\(^2\) and frozen in liquid nitrogen. The small intestine was removed and the first 2/3 starting from the pyloric valve was placed in ice-cold PBS.

Liver tissues were homogenised and hepatic microsomes were prepared by differential centrifugation using standard methods (Cinti et al., 1972). The preparation of intestinal microsomes was performed using a previously reported protocol (Leblond et al., 2002). Briefly, enterocytes were isolated from the first 2/3 of the small intestine by elution with an EDTA containing solution. Then, the cells were lysed and microsomes were prepared using two differential centrifugations at 12000 g and 100000 g. The pellets containing the microsomes were resuspended in 0.1 M Tris (pH 7.4), 20 % glycerol and 0.15 M NaCl and the microsomes obtained from each dose group were pooled. Protein concentration of hepatic and intestinal preparations was measured using bicinchoninic acid (BCA) as previously described (Smith et al., 1985).

**Measurement of the intrinsic clearance in intestinal and hepatic microsomes.**

Metoprolol was incubated at a final concentration between 0.5 and 200 µM for 30 min in 100 mM phosphate buffer (pH=7.4) containing 1.5 mM NADPH using 1 mg/mL of intestinal microsomes or 0.5 mg/mL of hepatic microsomes obtained from controls and ABT i.v. and p.o. pretreated rats. These conditions were optimized to be under linear turn-over conditions (results not shown). The reaction was terminated by the addition of acetonitrile containing 0.1 µg/mL of the internal standard labetalol.

**Gastric emptying and intestinal transit time study.**

The charcoal meal technique was used to evaluate the impact of ABT on the gastric emptying and the intestinal transit time. The protocol was designed based on published methods (Mittelstadt et al., 2005; Prior et al., 2012). Untreated rats and ABT i.v. and p.o.
pretreated rats (see Table 2 for details) were used for this experiment (n=4 per group). A control group pretreated 1h before with ABT p.o. was incorporated in the study to distinguish between the time of administration and the route of administration. A volume of 2 mL of charcoal meal (5 % (w/v) of activated charcoal in 2 % (w/v) of caboxymethyl-cellulose) was administered to the animals and 30 minutes later, the rats were anesthetized with isoflurane, sacrificed by cervical dislocation and the stomachs and the small intestines were collected. The full stomach was weighted and its content was removed by washing in phosphate buffer saline and then sponging with a gauze. The resulting empty stomachs were then weighted. The full length of the small intestine, from the pyloric valve to the ileo-caecal junction, and the maximal distance traveled by the charcoal from the pyloric valve to last visible charcoal were measured.

Sample preparation and analytical methods.

To determine the concentration of metoprolol and its major metabolites in plasma and microsomal incubates, samples were extracted by the addition of six volumes of acetonitrile containing the internal standard labetalol (0.1 ug/mL). The samples were vortexed and centrifuged at 2500 g for 11 minutes at 4 °C. The resulting supernatants were diluted 1:1 with water and analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

LC-MS/MS analysis was performed using a Thermo Fisher Scientific system composed of a TSQ Vantage mass spectrometer coupled with Accela U-HPLC system (Thermo Fisher). Chromatographic separation was achieved on an ACE 3 C18-PFP column (50 x 4.6 mm, from Advanced chromatography Technologies Ltd) using a linear gradient at 0.9 mL/min. Mobile phase A consisted of 0.1 % acetic acid in water and mobile phase B consisted of acetonitrile. A gradient program was initiated starting at 5 % B which was held for 0.3 minutes, then ramped from 5 % to 90 % B in 2.2 minutes. After holding at 90% B for an additional 1.2 minutes, the program changed back to 5% B. The column was equilibrated with 5% B for 1.2 minutes before
the next injection. The mass spectrometer was operated in the positive mode with electrospray ionization. The following transition along with their collision energy (CE) were used: 268.2 → 159.2 (CE : 20 V) for metoprolol, 254.1 → 177.1 (CE : 17 V) for ODM, 284.0 → 91.1 (CE : 38 V) for OHM, 268.2 → 145.2 (CE: 21 V) for MA and 329.1 → 311.2 (CE: 12 V) for labetalol.

**Data analysis.**

Pharmacokinetic parameters were determined by non-compartmental method using Watson (Thermo Scientific), a purpose-built laboratory information management system designed to support drug metabolism and pharmacokinetic studies. The area under the plasma concentration-time curve (AUC) from time 0 to last time point with a measurable concentration (AUC_{0-T}) was calculated by the trapezoidal method. The maximal plasma concentration (C_{max}), the half-life (T_{1/2}) and the time to reach C_{max} (T_{max}) were obtained from the observed data.

The kinetic constant K_{m} (the concentration at half the maximal activity) and V_{max} (the maximal velocity) were determined for each metabolite by fitting the data with the software Graphpad Prism using the nonlinear regression analysis. The CL_{int} for each metabolite was calculated by dividing the V_{max} by the K_{m}.

Gastric emptying was evaluated by the weight of the stomach content which is equal to the difference between the weight of the full stomach and the weight of the empty stomach. Intestinal transit time was measured by the % of the small intestine traveled by the charcoal and was calculated as follows: (distance traveled by the charcoal/small intestine length) x 100.

For group comparison, the statistical significance of the difference between mean values was calculated using analysis of variance (ANOVA) with Tukey-Kramer test used for multiple comparisons. Differences with a P value of less than 0.05 were considered to be statistically significant.
Results

Effect of ABT on Metoprolol oral pharmacokinetics in rats.

To evaluate the effect of pretreatment of ABT on the oral pharmacokinetics of metoprolol in rats, oral PK studies with metoprolol (10 mg/kg) were performed in control and ABT treated rats. The PK profiles of metoprolol and its major metabolites are represented in Figure 2 and the main PK parameters are shown in Table 3. Pretreatment with ABT p.o. significantly affected the PK of metoprolol as it increased the AUC by 16-fold and the Cmax by 5-fold versus untreated animals. In contrast to these finding, pretreatment with ABT i.v. resulted in a moderate increase in AUC (6.5-fold) and no effect on the Cmax of metoprolol. The inhibition of metoprolol metabolism by ABT is also confirmed by a decrease in the production of metabolites as measured by the ratio of the total AUC of the metabolites divided by the AUC of the parent compared to controls (from 81.2 in controls to 12.2 for ABT i.v. and 5.0 for ABT p.o.).

For the metabolites, both group pretreated with ABT increased the AUC of OHM and only ABT i.v. decreased the AUC of ODM. However, we found a significant reduction in the Cmax of the three metabolites following ABT pretreatment (i.v. and p.o.) compared to control, in line with a decrease in first pass metabolism. The decrease in the Cmax is more important in the group ABT i.v. compared to ABT p.o. for OHM and MA.

Moreover, a significant delay in absorption was observed in the ABT i.v. pretreated group (Tmax of 5.9 h) when compared to controls or ABT p.o. pretreated rats (0.25 and 0.5 h, respectively). This also translated to a delayed Tmax (up to 17-fold) of the three metabolites in the ABT i.v. pretreated group.
Effect of ABT on intestinal and liver metabolic enzymes.

To further put into perspective the PK data and elucidate the impact of pretreatment of ABT i.v. and ABT p.o. on liver and intestinal metabolism, microsomes were prepared from the liver and the intestine of untreated and ABT treated animals. The metabolic activity was assessed by measuring the CL_{int} of metoprolol in the microsomal preparation with the K_m and V_{max} method. Results are shown in Figure 3. In summary, ABT given orally or i.v inhibits significantly and to a similar extent the metabolism in intestinal and hepatic microsomes (5-fold and 3-fold, respectively). It is also worth mentioning that differences in metabolic pathways were observed in hepatic and intestinal microsomal incubations. Indeed, hepatic microsomes converted metoprolol mostly to MA and OHM while the intestinal microsomes produced mostly ODM.

Effect of ABT on gastric emptying and intestinal transit time.

To elucidate the potential effect of ABT i.v. and ABT p.o. on delaying absorption, gastric emptying and intestinal transit time were measured using the charcoal meal method. Four groups were studied: untreated, ABT i.v. 1 h before charcoal, ABT p.o. 16 h before charcoal and ABT p.o. 1 h before charcoal. Results are shown in Table 4. It is clear that a pretreatment of 1 h with ABT i.v. or p.o. significantly affects gastric emptying in rats as it increases by 7-fold the weight of the stomach content while a pretreatment of 16 h with ABT p.o. has no effect. Furthermore, no effect of ABT on intestinal transit was observed for all groups.

Discussion

The goal of the present study was to characterize the role of ABT in drug-drug interaction (DDI) studies by looking at its impact on absorption as well as its effect on intestinal and hepatic metabolism. This was achieved by conducting pharmacokinetics studies with
metoprolol under two well-defined ABT pretreatments. The three main metabolites of metoprolol were also monitored to corroborate the data obtained with the parent drug.

The oral pretreatment with ABT (16 h pre dosing) resulted in a large increase in the AUC and Cmax of metoprolol while ABT i.v (1 h pre-dosing) caused an increase of the AUC with no impact on Cmax (Table 3). In addition, the Cmax of the metabolites decreased compared to control. These results suggest that the inhibition of first pass metabolism is important under both ABT pretreatment conditions (i.v. and p.o.), with a stronger effect observed in the oral pretreatment group. This data could suggest that additional inhibition of intestinal enzymes is occurring when ABT is given orally, as proposed by Strelevitz et al., 2006. However, careful examination of the results may not support this hypothesis. Indeed, the decrease in the Cmax of the metabolites is more important in the ABT i.v. group even though the AUC of metoprolol increased less in this group compared to oral ABT group. Moreover, in the ABT i.v. group, the Tmax of metoprolol and its three metabolites are considerably delayed compared to control or ABT p.o. pretreated group suggesting a delayed absorption. Since the absorption is only delayed in the group ABT i.v., it is difficult then to conclude that the differences observed in the i.v. and p.o. groups is due exclusively to metabolism.

To clarify whether or not ABT i.v. inhibits intestinal metabolism, intestinal and hepatic microsomes were prepared from control and ABT-pretreated rats. The CLint of metoprolol measured in these microsomes is decreased similarly in the two pretreatment groups (5-fold and 3-fold in intestinal and hepatic microsomes, respectively) (Figure 3). These results clearly indicate that ABT cannot be used to discriminate between intestinal and liver first pass metabolism. In fact, based on the in vitro studies, both treatments inhibited more the intestine than the liver. In addition, it is not unusual to observe such an effect when a drug is dosed i.v. since the intestine is highly perfused (Dimich et al., 1991; Nakase et al., 2001)
Some differences were observed between the in vitro metabolism of metoprolol in rat intestinal and hepatic microsomes. Intestinal microsomes metabolize metoprolol almost exclusively in ODM while hepatic microsomes produced preferentially MA, followed by OHM and ODM. This difference can be explained by the specificity of cytochrome P450 isoforms as DDI studies with ketoconazole, cimetidine and quinidine performed in rats demonstrated that OHM and MA are produced by CYP2D and ODM by CYP3A (Boralli et al., 2009). As the rat intestine contains higher quantities of CYP3A versus CYP2D (Mitschke et al., 2008), this difference in expression levels will likely favor the formation of ODM in intestinal microsomes. This may explain the importance of the ODM and MA pathway in untreated rats as the intestine possibly produces a high amount of ODM that is later converted in MA by the liver. This can also explain the significant increase in the AUC of OHM following ABT p.o. pretreatment compared to control (Table 3). The intestine probably contribute less to the metabolism of metoprolol in ABT p.o. pretreated rats since it is more inhibited than the liver (Figure 3) and can only metabolize the drug during the first pass and not the elimination process. However, as the impact of ABT on the elimination of the metabolites themselves is unknown, a precise interpretation of these results remains unclear.

Since the inhibition of the intestine and the liver metabolism is similar in vitro for the ABT p.o. and ABT i.v. groups, we speculated that the delayed absorption observed in the ABT i.v. pretreated group may, in part, explain the difference in the PK of metoprolol between the ABT p.o. and i.v. groups. To better understand the impact of ABT on absorption, we evaluated gastric emptying and intestinal transit time with the charcoal meal technique (Table 4). A 1 h pretreatment with ABT (i.v. or p.o.) significantly inhibited the gastric emptying in rats. This effect is only temporary since no effect is in the 16 h pretreatment with ABT. In addition, it is worth noting that the charcoal meal technique can quickly and clearly distinguish between an effect on
gastric emptying or intestinal transit. Indeed, in the experiment with ABT, no effect on intestinal transit was observed.

These results are consistent with the study of Stringer et al, 2014 who demonstrates with a fluorescent marker that ABT inhibits the gastric emptying when dosed p.o. or intraperitoneally 2 h before the test meal. They observed no significant difference when ABT was dosed 15 h before the test meal in fasted male wistar rats. Therefore, we can conclude that ABT affects the gastric emptying when dosed 1-2 h before the experiment regardless of the route of administration and that this effect fades in time with no significant effect after 15-16 h.

The change in the absorption is of high importance considering that if the drug is liberated slowly from the stomach, the absorption will be delayed, the Cmax lowered and the Tmax delayed. Since less drug enters the circulation, the first pass extraction may also be increased. This is exactly what we observed in the present study: less metoprolol reaches the systemic circulation in the early time points in the i.v. ABT group likely due to the altered gastric emptying (Figure 2). This gives the false impression that the intestinal first pass metabolism is selectively inhibited in the ABT p.o. group. However, this is not the case as demonstrated by the in vitro studies where both p.o. and i.v. pretreatments gave similar reduced intestinal metabolic activity when compared to controls (Figure 3). Moreover, fewer metabolites also enter in the ABT i.v. group compared to ABT p.o. during the early time points which supports the conclusion that the absorption is altered (Figure 2). Thus, it is not possible to make conclusions about metabolism when using ABT if the rate of absorption is affected.

In conclusion, this study demonstrates that ABT cannot be used to distinguish between intestinal and hepatic first pass metabolism. Also, we have clearly shown that ABT can affect gastric emptying when rats are dosed 1-2 h prior to a pharmacokinetic study. For future studies, we recommend ABT to be dosed 15-16 h before the experiment (50 mg/kg p.o.). These
conditions are sufficient for the inhibition of intestinal and hepatic metabolic enzymes and do not affect the absorption phase. Therefore, ABT can still be a useful tool to determine if the cause of low oral bioavailability is due to phase I first-pass metabolism or poor absorption if it is correctly used.

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Authorship Contributions

Participated in research design: Boily, Chauret, Pichette, Laterreur, Leblond, Lévesque, Ste-Marie

Conducted experiments: Boily, Boudreau, Duquet

Performed data analysis: Boily

Wrote or contributed to the writing of the manuscript: Boily, Chauret, Pichette, Laterreur
References


Footnotes

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Figure Legends

Fig. 1. Metabolic pathway of metoprolol in rats. ODM is o-desmethyl-metoprolol, OHM α-hydroxy-metoprolol and MA metoprolol-acid (Borg et al., 1975a; Boralli et al., 2009).

Fig. 2. Plasmatic concentration of metoprolol (A), o-desmethyl-metoprolol (B), metoprolol-acid (C) and α-hydroxy-metoprolol (D) following the administration of 10mg/kg of metoprolol in untreated rats and in 50mg/kg ABT i.v. 1 h or ABT p.o. 16 h pretreated rats. Data are mean and standard deviations of five rats.

Fig. 3. Intrinsic clearance of metoprolol in hepatic (A) and intestinal (B) microsomes of untreated rats or ABT i.v. 1h and ABT p.o. 16 h pretreated rats. ODM is o-desmethyl-metoprolol, OHM α-hydroxy-metoprolol and MA metoprolol-acid.
### Tables

**Table 1.** Dosing regimen of ABT across different studies

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Route of administration</th>
<th>Time of pretreatment with ABT (hrs)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>p.o.</td>
<td>2</td>
<td>(Muzeeb et al., 2005; Sun et al., 2011)</td>
</tr>
<tr>
<td>50</td>
<td>p.o.</td>
<td>2</td>
<td>(El-Kattan et al., 2008; Subramanian et al., 2014)</td>
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<td>100</td>
<td>p.o.</td>
<td>15-16</td>
<td>(Strelevitz et al., 2006; Anby et al., 2014; Matsuda et al., 2014)</td>
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<tr>
<td>50</td>
<td>i.p.</td>
<td>16</td>
<td>(Caldwell et al., 2005)</td>
</tr>
<tr>
<td>50</td>
<td>i.v.</td>
<td>1</td>
<td>(Leong et al., 1997; Strelevitz et al., 2006).</td>
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Table 2. ABT pretreatment conditions prior to metoprolol dosing

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Dose volume (mL/kg)</th>
<th>Formulation</th>
<th>Time of pretreatment (hrs)</th>
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<td>1</td>
<td>Saline</td>
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<tr>
<td>ABT p.o.</td>
<td>50</td>
<td>5</td>
<td>Saline</td>
<td>16</td>
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</table>
Table 3. Effect of ABT on the PK parameters of metoprolol and its metabolites in rats

PK parameter after p.o. administration of 10 mg/kg of metoprolol in control or ABT treated rats. Data are mean and standard deviation of five rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>ABT i.v.</th>
<th>ABT p.o.</th>
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<tr>
<td><strong>Metoprolol</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC0-T (ug*h/mL)</td>
<td>0.166 ± 0.215</td>
<td>1.08 ± 0.37*</td>
<td>2.69 ± 0.77***###</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>0.250</td>
<td>5.90 ± 4.98*</td>
<td>0.500#</td>
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<tr>
<td>Cmax (ug/mL)</td>
<td>0.255 ± 0.296</td>
<td>0.233 ± 0.028</td>
<td>1.29 ± 0.37***###</td>
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<tr>
<td>T1/2 (h)</td>
<td>0.524 ± 0.141</td>
<td>3.98 ± 3.92</td>
<td>0.936 ± 0.199</td>
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<tr>
<td><strong>ODM</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>AUC0-T (ug*h/mL)</td>
<td>0.279 ± 0.114</td>
<td>0.0439 ± 0.0289***</td>
<td>0.183 ± 0.034#</td>
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<td>Tmax (h)</td>
<td>0.350 ± 0.137</td>
<td>6.10 ± 4.75*</td>
<td>1.30 ± 0.67#</td>
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<tr>
<td>Cmax (ug/mL)</td>
<td>0.138 ± 0.075</td>
<td>0.0224 ± 0.0058**</td>
<td>0.0561 ± 0.0153*</td>
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<tr>
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<td>1.39 ± 0.366</td>
<td>N.D.</td>
<td>2.00 ± 0.589</td>
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<tr>
<td><strong>OHM</strong></td>
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<td>AUC0-T (ug*h/mL)</td>
<td>1.05 ± 0.21</td>
<td>1.92 ± 0.32*</td>
<td>2.05 ± 0.77*</td>
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<tr>
<td>Tmax (h)</td>
<td>0.350 ± 0.137</td>
<td>6.10 ± 4.75*</td>
<td>0.500#</td>
</tr>
<tr>
<td>Cmax (ug/mL)</td>
<td>0.718 ± 0.229</td>
<td>0.169 ± 0.048*</td>
<td>1.29 ± 0.37***###</td>
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<td>27.6 ± 44.4</td>
<td>2.22 ± 0.366</td>
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<td><strong>MA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC0-T (ug*h/mL)</td>
<td>12.2 ± 1.1</td>
<td>11.3 ± 1.3</td>
<td>11.3 ± 0.7</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>1.00</td>
<td>6.40 ± 4.34*</td>
<td>1.80 ± 0.45#</td>
</tr>
<tr>
<td>Cmax (ug/mL)</td>
<td>4.86 ± 1.00</td>
<td>1.02 ± 0.18***</td>
<td>3.16 ± 0.44***###</td>
</tr>
<tr>
<td>T1/2 (h)</td>
<td>2.78 ± 0.513</td>
<td>14.5 ± 24.4</td>
<td>2.60 ± 1.39</td>
</tr>
</tbody>
</table>

*a Rats were treated i.v. with 50 mg/kg of ABT 1 h before the administration of metoprolol

*b Rats were treated p.o. with 50 mg/kg of ABT 16 h before the administration of metoprolol

Statistically significant differences: * P<0.05, ** P<0.01, *** P<0.001 ABT i.v. and ABT p.o. versus control; # P<0.05, ## P<0.01, ### P<0.001 ABT i.v. versus ABT p.o.
Table 4. Effect of ABT on stomach content and intestinal transit in rats dosed with a charcoal meal

The stomach content (g) and the intestinal transit (% of the small intestine travelled by the charcoal) were measured 30 minutes after the administration of 2 mL of activated charcoal in control or 50 mg/kg ABT treated rats. Data are mean and standard deviation of four rats.

<table>
<thead>
<tr>
<th>Stomach content (g)</th>
<th>Intestinal transit (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>0.464 ± 0.063</td>
</tr>
<tr>
<td>ABT i.v. 1 h before charcoal</td>
<td>3.21*** ± 0.49</td>
</tr>
<tr>
<td>ABT p.o. 16 h before charcoal</td>
<td>0.658 ± 0.510</td>
</tr>
<tr>
<td>ABT p.o. 1 h before charcoal</td>
<td>3.33*** ± 0.86</td>
</tr>
</tbody>
</table>

Statistically significant differences: * P < 0.05, ** P < 0.01, *** P < 0.001 versus control
Figure 1

Metoprolol → O-Dealkylation → ODM

Hydroxylation → OHM

Oxidation → MA
Figure 3

A

B

Control
ABT i.v.
ABT p.o.

Clearance (μL/min/mg)

ODM
OHM
MA
Total

ODM
OHM
MA
Total